Legume responses to low phosphorus fertility

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INTRODUCTION

A variety of low-molecular weight organic compounds inhabit the spaces with soils and bathe the roots of plants. These chemicals serve in communication in plant-animal relations, and solubilize slowly-available nutrients from soils. There is much interest in their distribution and transport across soil-water-root interfaces. We exposed several mung bean (*Vigna mungo* L.) plants over 1 –15 weeks to either nutrient-replete conditions or low-phosphorus. Plants are sown directly into "rhizoboxes" of dimensions 20 cm x 10.5 cm x 1 cm thick, and a portion of the roots exposed to the mid-IR synchrotron light of ALS Beamline 1.4.3. The IR spectra are acquired from the root/soil interface through a 50 mm x 20 mm x 1 mm thick zinc selenide trapezoidal window (Spectral Systems Inc.; Hopewell Junction NY). Chemically detailed and fine-spatial scale IR reflection spectra are compared to discern root responses to abiotic stressors in wild and cultivated members of the legume plant family. Low-phosphorus availability was the first challenge of our model edible legume, *Vigna mungo* L. In other experiments, we collected spectra from the wild desert legume *Lupinus arizonicus* L. to compare responses between species.

A second, parallel goal of the research is to identify spectral features useful in measuring diffusion coefficients with IR microscopy. Amino acids are important soil resources of N and C to plants and soil microorganisms [1,2], and their availability is likely to increase under projected future atmospheric CO_2 levels. Thus it is necessary to estimate their diffusion coefficients in realistic substrates. In the last two years of experiments we grew plants in acid-washed silica/feldspar sand of particle diameter > 250 μm. In preliminary experiments in 1998 to screen suitable solid media for root studies at Beamline 1.4.3, we discovered that high organic matter soils from Colorado and Hawai'i have too little reflected IR signal for spectromicroscopy.

RESULTS FOR THIS YEAR

In **Figure 1**, you see typical IR reflection spectra acquired from the root zone of mung bean (*Vigna mungo* L.) seedlings exposed to either low-phosphorus(A) or nutrient-complete (B) conditions. The plants were cultured on ¼-strength modified Hoagland's Solution, with micronutrients and NH_4^+:NO_3^- ratio appropriate for legumes. Seeds were sown directly in the root boxes, and the mung bean plants subsequently expressed large branching root systems, a portion of which was coincident with the ZnSe IR-transmitting windows. Spectra are acquired from 4500 – 650 cm^{-1}, since the low energy cut-off for this window material is 650 cm^{-1}.

Infrared reflectance spectra obtained of the root zone through the Nicolet 760 FT-IR spectrometer/microscope and a 15x objective demonstrated significant differences in mung beans exposed to low-P conditions as compared to the nutrient-sufficient plants **(Fig 1 a, b)**. Spectra acquired in below 1600 cm^{-1} in the mid-infrared is known as the 'fingerprint region', and particularly noticeable are strong IR absorption features between 1050 and 700 cm^{-1} in the low-P treated mung beans. These features match ester absorbances of polysaccharide skeletons. With complete nutrients, absorbance features shifted to higher energies, a cluster between 1300 and 950 cm^{-1} particularly evident. These spectral features may be attributed to C-O stretching of aromatic and aliphatic carboxylic anhydrides. Small white crystalline inclusions were periodically observed in the root-zone of low-phosphorus mung beans, though not in nutrient-replete plants. IR spectra (data not shown) collected across one of these inclusions (at 10 micron spatial resolution) matched the library spectra for gypsum, $CaSO_4$. As one moved from the center of a crystal to its edge, one observes a complex organic film, the very surface of which contains organic-phosphorus compounds overlying the gypsum skeleton. Such precipitates may serve as biological "nucleation" centers for the growth of microbes.

Common to all plants examined are water vapor features from 4000 to 3500 cm^{-1} and 2000 - 1400 cm^{-1}, which show extensive rotational fine-structure expected for compounds in the gas phase. Common as well to all root spectra is the strong IR doublet of CO_2 at 2360 cm^{-1}. This CO_2 arises from both root and heterotrophic respiration within the soil microcosms, as ambient atmospheric levels are suppressed by the dry nitrogen apron covering the IR window region below the microscope objectives. A useful mapping wavelength for root tissue is the doublet at 3000 - 2900 cm^{-1}, arising from bending modes of aliphatic C-H bonds in surface suberin layers of root tissue [3].

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ALS experimental research is supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Science Division, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. I also acknowledge the support of the Department of Aerospace Sciences, BioServe Space Technologies, Boulder CO.

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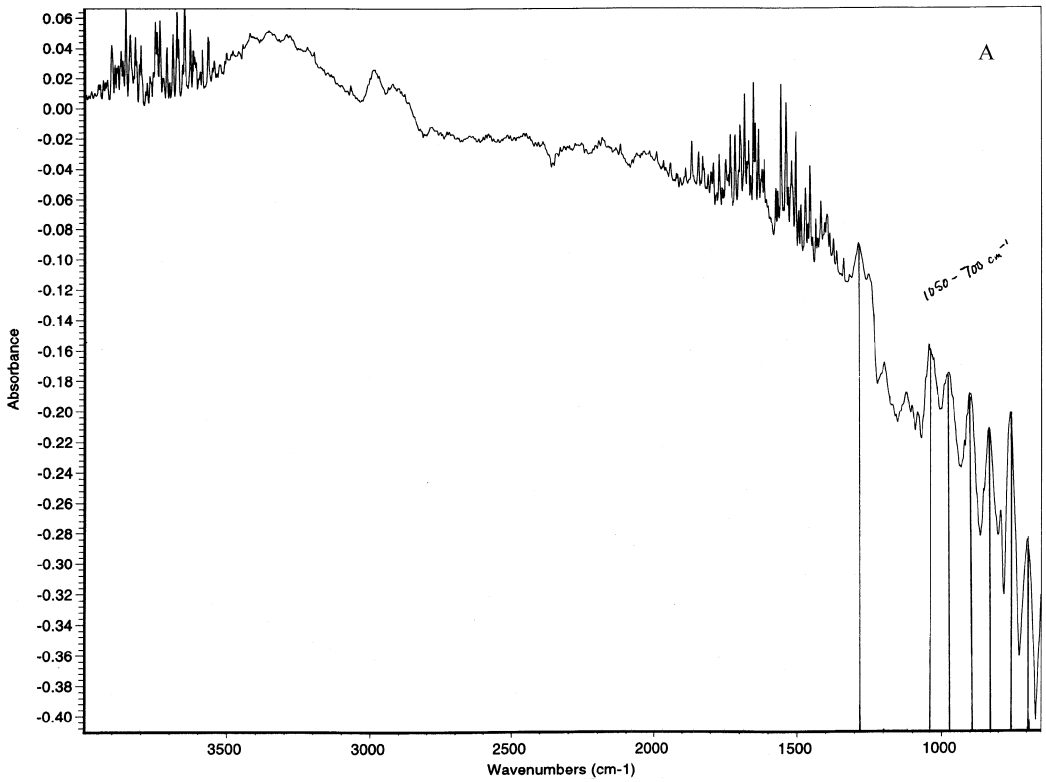

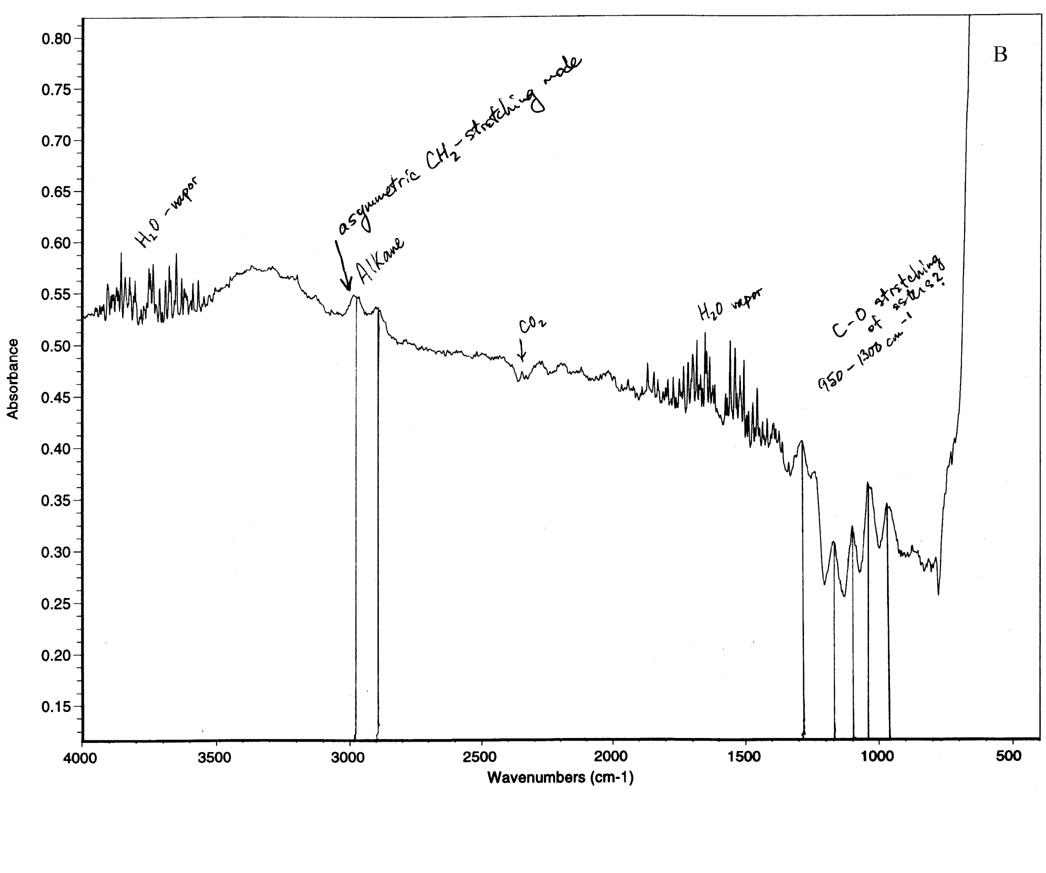

Figure 1: Mid-infrared reflection spectra collected of roots and associated exudates from 42 day old mung bean plants exposed to low-phosphorus (A) conditions and (B) complete nutrients. Spectra represent the average of 512 transients